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# Using multifractal analysis of ultra-weak photon emission from germinating wheat seedlings to differentiate between two grades of intoxication with potassium dichromate

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**Abstract**. The aim of the present study was to test whether the multifractal properties of ultra-weak photon emission (UPE) from germinating wheat seedlings (*Triticum aestivum*) change when the seedlings are treated with different concentrations of the toxin potassium dichromate (PD). To this end, UPE was measured (50 seedlings in one Petri dish, duration: approx. 16.6–28 h) from samples of three groups: (i) control (group C, $N = 9$), (ii) treated with 25 ppm of PD (group $G_{25}$, $N = 32$), and (iii) treated with 150 ppm of PD (group $G_{150}$, $N = 23$). For the multifractal analysis, the following steps where performed: (i) each UPE time series was trimmed to a final length of 1000 min; (ii) each UPE time series was filtered, linear detrended and normalized; (iii) the multifractal spectrum ($f(\alpha)$) was calculated for every UPE time series using the backward multifractal detrended moving average (MFDMA) method; (iv) each multifractal spectrum was characterized by calculating the mode ($\alpha_{mode}$) of the spectrum and the degree of multifractality ($\Delta\alpha$); (v) for every UPE time series its mean, skewness and kurtosis were also calculated; finally (vi) all obtained parameters where analyzed to determine their ability to differentiate between the three groups. This was based on Fisher's discriminant ratio (FDR), which was calculated for each parameter combination. Additionally, a non-parametric test was used to test whether the parameter values are significantly different or not. The analysis showed that when comparing all the three groups, FDR had the highest values for the multifractal parameters ($\alpha_{mode}$, $\Delta\alpha$). Furthermore, the differences in these parameters between the groups were statistically significant ($p < 0.05$). The classical parameters (mean, skewness and kurtosis) had lower FDR values than the multifractal parameters in all cases and showed no significant difference between the groups (except for the skewness between group C and $G_{150}$). In conclusion, multifractal analysis enables changes in UPE time series to be detected even when they are hidden for normal linear signal analysis methods. The analysis of changes in the multifractal properties might be a basis to design a classification system enabling the intoxication of cell cultures to be quantified based on UPE measurements.

## 1. Introduction

Ultra-weak light (~ $10^2$ photons/s $cm^2$ [51]) in the wavelength range of approx. 200–800 nm is spontaneously emitted from the surface of every living organism. This ultra-weak photon emission (UPE) is generally considered to be ultra-weak chemiluminescence caused by the formation of energetically excited products ($P^*$) from the reactants K and M (which could be atoms or molecules), followed by a relaxation of $P^*$ to a deeper energy level, accompanied by the emission of photons: $K + M \rightarrow P^* \rightarrow P + hv$ [1]. The excited states are considered to be mainly caused by interactions with reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) that are produced under physiological states (e.g. by mitochondria in the respiratory chain [35], by phagocytic cells during the "respiratory burst" [14] or by ionizing radiation from internal and external sources [17, 18, 20, 19]) and pathophysiological states (e.g. during postischemic reperfusion [3]). The interaction of ROS/RNS with biomolecules leads to exothermic reactions where photons are emitted, e.g. during the interaction of ROS/RNS with lipids [4, 42] or proteins [43]. On the other hand, electronically excited states and their relaxation are caused by mechanisms other than those based on ROS/RNS. For example, Tuszyński and Dixon [46] showed that the activity of the proton pump in the mitochondrial wall could be a source of UPE as a consequence of the excitation and relaxation of cytochrome enzymes. Additionally, the reaction of triplet oxygen ($^3O_2$) with bilirubin under aerobic conditions and in an alkaline solutions exhibits UPE (with a peak at ~ 670 nm) [52]. Furthermore, non-enzymatic amino-carbonyl reactions between reducing sugars and amino acids (Maillard reactions) cause UPE (with two broad peaks at ~ 500 nm and ~ 695 nm) [53]. These reactions take place in biological systems as a step in the formation of advanced glycation end-products (AGEs) [32]. Thus, while ROS/RNS reactions seem to be the primary source of UPE, other physiochemical processes also appear to be involved.

The physiological significance of UPE is an ongoing debate. While on the one hand it is proven that UPE is correlated with physiological and pathophysiological states (e.g. temperature [10, 38], cell cycle state [27, 31, 5], "respiratory burst" during immune reactions [54], cell density [41], normal vs. cancer tissue [36], physical exercise [28], chronobiological phase [9, 8, 7, 49, 48, 23, 11], growth dynamics of germinating seedlings [24, 25, 47]), the role of UPE as an active factor regulating biological processes is still under investigation on the other hand [50, 6].

Previous experiments revealed that intensity and dynamics of UPE from germinating wheat seedlings are altered by intoxication with potassium dichromate (PD) ($K_2Cr_2O_7$) of different concentrations. PD is a oxidative stress causing [40, 44], carcinogenic [39] and genotoxic [12] agent that can readily cross cellular membranes. It was found that (i) UPE increased with very mild PD intoxication by 1.5 ppm [33] and 15 ppm [33, 2] compared to the UPE intensity from the control group. Also (ii) the UPE dynamics showed enhanced oscillations with a 6 h period length under PD intoxication of 1.5 ppm [2].

The aim of the present study was to further investigate the effects of the treatment of germinating wheat seedling with differing PD concentrations on UPE dynamics. In particular, we investigated whether it is possible to differentiate between three different groups of wheat seedlings ($G_{25}$: treated with 25 ppm of PD; $G_{150}$: treated with 150 ppm of PD; C: control) based on the analysis of the UPE dynamics. To this end, we used multifractal time series analysis to investigate the UPE dynamics. To the best of our knowledge, the use of multifractal analysis to analyze UPE signals is reported here for the first time.

## 2. Material and methods

### *2.1. Sample preparation and UPE measurements*

Three groups of wheat seedling (*Triticum aestivum*) were prepared in Petri dishes, containing 50 wheat grains in 10 mL solution each: (i) control (group C, $N = 9$), (ii) treated with 25 ppm of PD (group $G_{25}$, $N = 32$), and (iii) treated with 150 ppm of PD (group $G_{150}$, $N = 23$). After the samples had been kept in a dark chamber with controlled temperature (20 °C) for 48 h, UPE was measured using a photomultiplier tube (Hamamatsu PMT H7360, spectral response range: 300-650 nm, peak sensitivity

wavelength: 375 nm, dark noise: 15 cps) and a counting board (Hamamatsu M8784). Every measurement was started at the same time (9:30 am) and for a time span in the range of 16.6–28 h. The UPE values were acquired in 10 s time slots.

*2.2. Multifractal detrended moving average (MFDMA) method*

The rationale behind the multifractal approach is that a single scaling exponent (the fractal dimension $d_f$) is often not enough to describe the whole complex dynamics of a given non-stationary time series. To overcome this problem, the framework of multifractal analysis was developed based on the multifractal formalism introduced by Frisch and Parisi [16], Halsey et al. [22] and Mandelbrot [30]. Multifractal analysis allows the multiply scaling laws to be quantified by calculating the multifractal spectrum ($f(\alpha)$) which is a representation of the multifractal scaling of the time series. $f(\alpha)$ can be calculated by different approaches, e.g. the multifractal box-counting (MF-BOX) method [13], the wavelet transform modulus maxima (WTMM) method [37], the multifractal detrended fluctuation analysis (MFDFA) [26], the wavelet leader (WL) based multifractal analysis [29], the partition function and structure function method [15], or the multifractal detrended moving average (MFDMA) method [21]. Since it was shown that MFDMA is superior to MFDFA in quantifying the multifractality [21], MFDMA was used in the present study. A detailed mathematical description of the MFDMA method can be found in the paper of Gu and Zhou [21].

*2.3. Data analysis*

For the data analysis, the following steps where performed: (i) each UPE time series was trimmed to a final length of 1000 min (starting 100 s after the beginning of the measurement); (ii) each UPE time series was filtered (by applying a Savitzky-Golay filter (span: 3 samples) to remove the noise), linear detrended and normalized by scaling to the range [0, 1]; (iii) the multifractal spectrum was calculated for every UPE time series; (iv) each multifractal spectrum was characterized by calculating the mode ($\alpha_{mode}$) of the spectrum and the degree of multifractality ($\Delta\alpha = \alpha_{max}–\alpha_{min}$); (v) for every UPE time series, its mean, skewness and kurtosis were also calculated; finally (vi) all obtained parameters where analyzed for their capability to separate the three groups by calculating the Fisher's discriminant ratio (FDR) for each parameter combination. FDR is a measure of the group discriminability (the higher the FDR value the higher the discriminability power). FDR is defined as [45]

$$FDR = \frac{(m_1 - m_2)}{(\sigma_1^2 - \sigma_2^2)} \quad (1)$$

where $m_1$ and $m_2$ are the mean values of the two distributions and ${\sigma_1}^2$ and ${\sigma_2}^2$ the respective variances. In addition to FDR, a non-parametric test was used (Wilcoxon test) to test whether the parameter values are significantly different or not.

For the calculation of the multifractal spectrum, the following values for the lower bound of the segment size $n$ ($n_{min}$), upper bound of the segment size $n$ ($n_{max}$), the length of $n$ ($N$), the multifractal order ($q$) were used according to the suggestion of [21]: $n_{min} = 10$; $n_{max} = 10\%$ of the length of the time series used; $N = 30$, $q = [-4, 4]$. Additionally, we used a backward detrending window in MFDMA since it was shown that it was superior to the usage of a centered or forward detrending window [21].

## 3. Results

The multifractal analysis of the UPE signals revealed that the characteristics of the multifractal spectra were different for each group (see Fig 1-2, Tab 1). When comparing all the three groups, FDR had the highest values for the multifractal parameters ($\alpha_{mode}$, $\Delta\alpha$); also, the differences of these parameters between the groups where statistically significant ($p < 0.05$) (see Fig 2, Tab 1). The classical parameters (mean, skewness and kurtosis) had lower FDR values than the multifractal parameters in all cases and showed no significant difference between the groups – except for the skewness between group C and $G_{150}$ (see Fig 2, Tab 1).

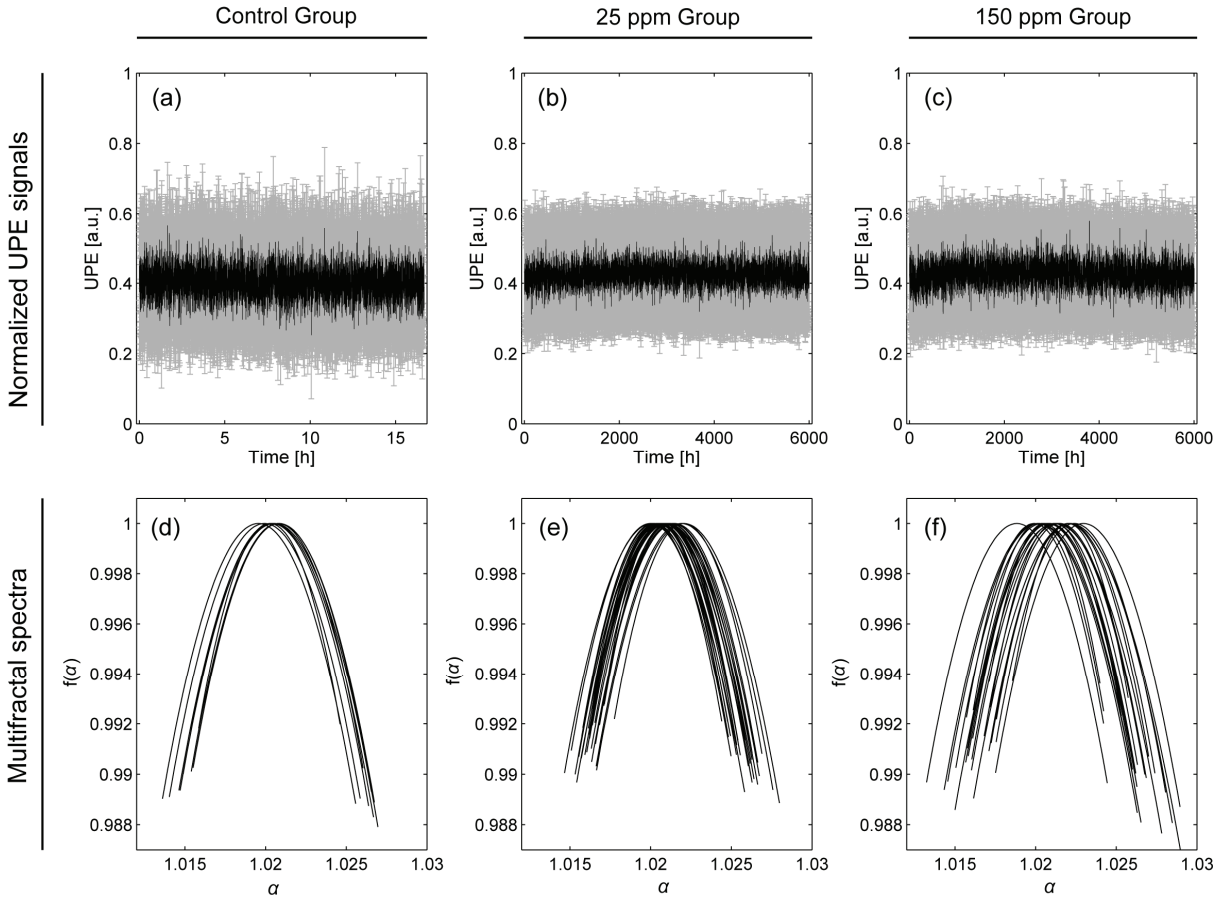


**Figure 1.** Results of the multifractal analysis. Subfigures (a)–(c): detrended and normalized UPE signals of the three groups (black lines: mean, grey: standard deviation), (d)–(f) corresponding multifractal spectra. It is clear that the distribution of the multifractal spectra is different for the 150 ppm and 25 ppm group compared to the control group.

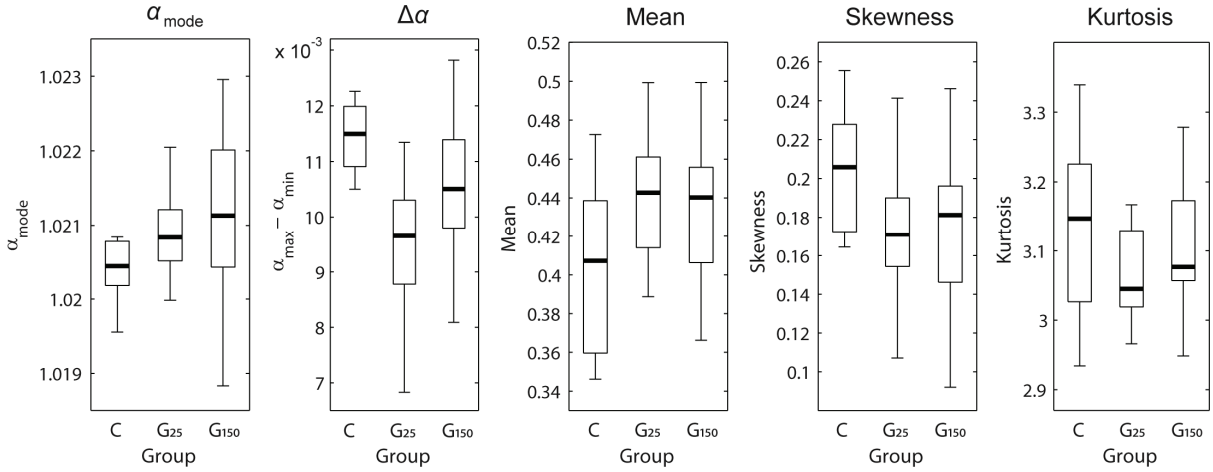


**Figure 2.** Boxplots of the determined values of multifractal parameters ($\alpha_{mode}$, $\Delta\alpha$) and classical parameters (mean, skewness and kurtosis).

**Table 1**. Results of the statistical analysis. The asterisks refer to the significance levels: significant (*, $p < 0.05$), highly significant (***, $p < 0.001$).

| Parameter | C vs. $G_{25}$ | | C vs. $G_{150}$ | | $G_{25}$ vs. $G_{150}$ | |
|---|---|---|---|---|---|---|
| | *FDR* | *p* | *FDR* | *p* | *FDR* | *p* |
| $\alpha_{mode}$ | 0.4585 | 0.0326 * | 0.5099 | 0.0244 * | 0.0408 | 0.3106 |
| $\Delta\alpha$ | 0.2573 | 0.0715 | 1.2262 | 0.0006339 *** | 0.1425 | 0.0267 * |
| Mean | 0.2135 | 0.1021 | 0.1648 | 0.0643 | 0.0000896 | 0.5965 |
| Skewness | 0.3599 | 0.0539 | 0.3682 | 0.0343 * | 0.0000551 | 0.8505 |
| Kurtosis | 0.0463 | 0.5025 | 0.0001349 | 0.2237 | 0.0138 | 0.3106 |

## 4. Discussion, conclusion and outlook

The results of the data analysis show that the multifractal parameters $\alpha_{mode}$, $\Delta\alpha$ had the largest FDR values, indicating that they can be used to differentiate between the UPE time series originating from the three sample groups of germinating wheat seedlings. The differences observed in the multifractal parameters indicate that the UPE time series show different non-linear characteristics of their dynamical behavior with respect to the state of the germinating seedlings (not stressed [C], mildly intoxicated [$G_{25}$], and strongly intoxicated [$G_{150}$]).

The interpretation of the multifractal parameters $\alpha_{mode}$, $\Delta\alpha$ is as follows: the local Hölder exponent $\alpha_{mode}$ is related to the degree of irregularity of the analyzed signal (the lower $\alpha_{mode}$, the more irregular the signal), $\Delta\alpha$ reflects the strength of multifractality (the higher $\Delta\alpha$, the more structure has the signal) [21, 34]. Based on these facts and based on the results obtained for the multifractal parameters (see Fig 2), we conclude that the more stressed the germinating wheat seedlings are, the more irregular and less structured the UPE time series become. Interestingly, the strength of multifractality $\Delta\alpha$ decreases more for the intoxication with 25 ppm PD than with 150 ppm.

In conclusion, multifractal analysis enables the detection of changes in UPE time series that are hidden for normal linear signal analysis methods. The non-linear features of a UPE time series seem to be related to the states of the bio-system. The analysis of changes in the multifractal properties might be a basis to design a classification system enabling the intoxication of cell cultures to be quantified based on UPE measurements. Further investigations also have to address the questions of how different parameters of (i) the UPE data (i.e. the length of the time series), (ii) the preprocessing process (i.e. type of applied filter, span of the filter, type of detrending method, type of normalization), and (iii) the determination of the multifractal parameters (i.e. type of method, type of characterization of the multifractal spectra) influence the results obtained, and how they could be optimized to improve the classification of the UPE time series based on multifractal analysis.

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